

functions carried out by PKD that can best be explained by a new model that requires specific spatially-resolved subcellular targeting.

### 3210-Pos Board B257

#### Higher Aromatase Expression In Female Heart May Underline Its High Estrogen Content Resulting In Cardioprotection

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Estrogen (E2) is a well-known cardioprotective steroid hormone. Although heart has all the machinery to biosynthesize estrogen from testosterone by cytochrome P450 aromatase, little is known about the role of local heart E2 concentration [E2] in cardioprotection. We hypothesized that high heart [E2] in females could be one mechanism for the higher cardioprotection in females. We optimized the radioimmunoassay technique to measure heart [E2] in whole homogenate by diethyl ether extraction. Male mouse hearts have significantly higher E2 levels ( $35 \pm 3$  pg/ml,  $n=6$ ) than plasma ( $12 \pm 0.9$  pg/ml,  $n=5$ ). Heart [E2] in female mice at estrus and diestrus (diestrus  $20.2 \pm 1.5$  pg/ml  $n=4$ ; estrus  $17.2 \pm 0.9$  pg/ml,  $n=4$ ) were very similar to plasma [E2]. Interestingly, in the proestrus stage, heart [E2] was extremely high  $\sim 170 \pm 4$  pg/ml, almost 3 times higher than plasma [E2]. The final heart [E2] will depend on the testosterone level as well as the efficiency of the aromatase to convert testosterone to E2. As females have much lower levels of testosterone ( $\sim 40$  pg/ml at estrus and diestrus and  $\sim 240$  pg/ml in proestrus) compared to males (2 ng/ml), much higher heart [E2] in females at proestrus compared to male lead us to hypothesize that the aromatase expression/activity is much higher in females than males. We performed real time PCR and western blot analysis to quantify transcript and protein levels of aromatase in male and in female mice at estrus stage, as this stage is under the control of the preceding estrogen peak at proestrus. Aromatase transcript levels were similar in males and females at estrus, but aromatase protein levels were two fold higher in estrus compared to male. We speculate higher aromatase expression in females may underline its high estrogen content, thus resulting in cardioprotection.

### 3211-Pos Board B258

#### Glyceollin Attenuates Vascular Contraction By Inhibiting RhoA/rho Kinase Pathway

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Isoflavones such as genistein and daidzein prevented agonist-induced vascular contraction in isolated rat aortic rings. Glyceollins are derived from the parent isoflavone daidzein through a series of pterocarpin intermediates. We hypothesized that glyceollin attenuates vascular contractions through inhibition of RhoA/Rho kinase pathway. Rat aortic rings were denuded of endothelium, mounted in organ baths and treated with either glyceollin (20 or 100  $\mu$ M) or vehicle (DMSO) for 60 min after submaximal contraction by NaF (8.0 mM). The phosphorylation level of the myosin light chain (MLC<sub>20</sub>), myosin phosphatase target subunit 1 (MYPT1) and protein kinase C (PKC)-potentiated inhibitory protein for heterotrimeric myosin light chain phosphatase of 17-kDa (CPI17) were determined by means of the Western blot. Glyceollin not only inhibited vascular contractions induced by NaF (8.0 mM), but also decreased the activation of RhoA and subsequent phosphorylation of MYPT1<sup>Thr855</sup> and CPI17<sup>Thr38</sup>. These results indicate that glyceollin attenuates vascular contraction by inhibiting RhoA/Rho-kinase signaling.

### 3212-Pos Board B259

#### 17 $\beta$ -Estradiol Attenuates Vascular Contraction Through Inhibition Of RhoA/Rho Kinase Signaling Pathway

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It is now well-known that 17 $\beta$ -estradiol has an endothelium-independent, non-genomic vasorelaxant action. In the present study, we hypothesized that 17 $\beta$ -estradiol attenuates vascular contraction by inhibiting RhoA/Rho kinase signaling pathway in rat aorta. Rat aortic rings were denuded of endothelium, mounted in organ baths, and contracted with 30 nM U46619 or 8.0 mM NaF 30 min after pretreatment with 17 $\beta$ -estradiol (30 and 100  $\mu$ M) or vehicle. We measured the amount of GTP RhoA and the level of phosphorylation of the myosin light chain (MLC<sub>20</sub>), myosin phosphatase targeting subunit 1 (MYPT1) and PKC-potentiated inhibitory protein for heterotrimeric MLCP of 17 kDa (CPI17). Pretreatment with 17 $\beta$ -estradiol not only inhibited U46619- or NaF-induced vasocontractions and the phosphorylation of

MLC<sub>20</sub> but also inhibited activation of RhoA. 17 $\beta$ -Estradiol also decreased the level of phosphorylation of MYPT1<sup>Thr855</sup> and CPI17<sup>Thr38</sup>, downstream effectors of Rho-kinase. In conclusion, 17 $\beta$ -estradiol attenuates vascular contraction, at least in part, through inhibition of RhoA/Rho kinase signaling pathway.

**Key Words:** 17 $\beta$ -estradiol, RhoA, Rho kinase, CPI-17, MYPT1, vasorelaxation

### 3213-Pos Board B260

#### Modulation Of Cardiac Na<sup>+</sup>/H<sup>+</sup> Exchange Activity By Muscarinic Agonists, Nitric Oxide and Cyclic GMP

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Na<sup>+</sup>-H<sup>+</sup> exchange (NHE) is the principal acid-extrusion mechanism in cardiac myocytes. Its activity has been linked to myocardial ischaemia-reperfusion injury, arrhythmia and the development of cardiac hypertrophy. NHE is modulated acutely by intracellular pH (pH<sub>i</sub>), as well as through phosphorylation by kinases. Nitric oxide (NO) is an important regulator of cardiac function. It is synthesised by NO synthases (NOS), which are activated by muscarinic (M<sub>2</sub>) receptors. NO targets proteins, partly via protein kinase G, which is activated by cyclic GMP (cGMP). We studied the effect of this regulatory pathway on NHE activity in rat ventricular myocytes. Myocytes were loaded with the acetoxymethyl-ester of carboxy-SNARF-1 (a pH-reporter dye) and superfused with Hepes-buffer at 37°C. Applying a 4min, 20mM NH<sub>4</sub>Cl prepulse deposits an intracellular acid-load that stimulates NHE. The membrane-permeant cGMP analog 8Br-cGMP (20 $\mu$ M), the NO donor sodium nitroprusside (1mM) and the M<sub>2</sub> agonist carbachol (100 $\mu$ M) reduced NHE activity (vs paired controls). At a common pH<sub>i</sub> of 6.6, NHE inhibition was 26%, 29% and 18%, respectively ( $P > 0.05$ ). We also transfected adult myocytes with the nNOS gene using an adenoviral system ( $5 \times 10^{10}$  viral particles, incubated overnight) to increase NO production capacity. To confirm successful gene-transfer, eGFP was transfected in separate experiments and fluorescence was detected in >90% of cells. NHE activity at pH<sub>i</sub>=6.6 was not significantly different in nNOS-transfected cells (vs sham-transfected cells). However, on addition of carbachol (100 $\mu$ M), NHE activity was reduced by 50%. These findings illustrate an important role for the NO/cGMP pathway in modulating pH<sub>i</sub> homeostasis.

Work supported by the British Heart Foundation and Wellcome Trust.

### 3214-Pos Board B261

#### Colocalization Of RyR And Ca<sub>v</sub>1.2 In Ventricular Myocytes Is Independent Of The Physical Orientation Of The Cell

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We have investigated the effect of cell orientation on the observed colocalization of the ryanodine receptor (RyR) with the L-type calcium channel (Ca<sub>v</sub>1.2) in adult rat ventricular myocytes. Cells were embedded in 2% agarose<sup>1</sup> and visualised with a 60X 1.2 NA water immersion objective on an Olympus FV1000 confocal microscope; all images were deconvolved before analysis. We imaged cells oriented both parallel and perpendicular to the coverslip. We found that colocalization between RyR and Ca<sub>v</sub>1.2 in the two orientations was not significantly different from each other, and similar to values previously reported<sup>2</sup>. Cells oriented perpendicular to the coverslip provided additional details of the colocalization: The colocalized region between RyR and Ca<sub>v</sub>1.2 was often surrounded by an area of RyR fluorescence, implying that the calcium channels within a dyad cover a smaller area than do the RyR. RyR that had no corresponding Ca<sub>v</sub>1.2, and are presumed to be extra-dyadic, were distributed in the Z disk with no discernable pattern.

1. Chen-Izu, Y, et al. (2006) Biophys. J 91: 1-13.

2. Scriven, DR, et al. (2000) Biophys. J. 79: 2682-2691.

### 3215-Pos Board B262

#### Blocking the Late Sodium Current Reduces Intracellular Sodium Accumulation During Sodium Pump Inhibition

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Impairment of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity reduces sodium efflux and leads to an increase in intracellular Na<sup>+</sup> (Na<sup>+</sup><sub>i</sub>). We tested the hypothesis that Na<sup>+</sup> accumulation caused by ouabain, a Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitor, would be reduced by concurrent inhibition of the late sodium current (I<sub>NaL</sub>). We measured Na<sup>+</sup><sub>i</sub>, high energy phosphates, and chemical driving force ( $\Delta G_{ATP}$ ) in isolated guinea pig hearts in real time with <sup>23</sup>Na- and <sup>31</sup>P- NMR. Hearts were pre-